Suitability of Two Rainbow Trout (*Salmo gairdneri*) Reference Diets for Arctic Charr (*Salvelinus alpinus*)

M. Yurkowski

Western Region
Department of Fisheries and Oceans
Winnipeg, Manitoba R3T 2N6

August 1986

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1464

86-05960/

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in *Aquatic Sciences and Fisheries Abstracts* and indexed in the Department's annual index to scientific and technical publications.

Numbers 1–456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457–714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715–924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page. Out-of-stock reports will be supplied for a fee by commercial agents.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. Il n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques du ministère des Pêches et des Océans, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications complètes. Le titre exact paraît au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la revue *Résumés des sciences aquatiques et halieutiques*, et ils sont classés dans l'index annual des publications scientifiques et techniques du Ministère.

Les numéros 1 à 456 de cette série ont été publiés à titre de rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre. Les rapports épuisés seront fournis contre rétribution par des agents commerciaux.

Canadian Technical Report of
Fisheries and Aquatic Sciences 1464

August 1986

SUITABILITY OF TWO RAINBOW TROUT (*Salmo gairdneri*) REFERENCE DIETS FOR ARCTIC CHARR (*Salvelinus alpinus*)

by

M. Yurkowski

Western Region
Department of Fisheries and Oceans
Winnipeg, Manitoba R3T 2N6

This is the 198th Technical Report
from the Western Region, Winnipeg

© Minister of Supply and Services Canada 1986

Cat. no. Fs 97-6/1464E ISSN 0706-6457

Correct citation for this publication is:

Yurkowski, M. 1986. Suitability of two rainbow trout (Salmo gairdneri) reference diets for Arctic charr (Salvelinus alpinus). Can. Tech. Rep. Fish. Aquat. Sci. 1464: iv + 10 p.

TABLE OF CONTENTS

	Page
ABSTRACT/RÉSUMÉ	iv
INTRODUCTION	1
PROCEDURES	1
RESULTS AND DISCUSSION	2
Practical reference diet C201	2
Semipurified reference diet C101	2
SUMMARY AND CONCLUSIONS	3
ACKNOWLEDGMENTS	3
REFERENCES	3

LIST OF TABLES

Table		Page
1	Practical reference diet (C201) formulation	5
2	Semipurified reference diet (C101) formulation	6
3	Performance and mortality of Arctic charr fed semipurified refernce diet C101 and practical reference diet C201	7
4	Moisture, lipid, fat-free solid, protein, riboflavin niacin and thiamine content of diets, livers and whole bodies of Arctic charr fed sumipurifed reference diet C101 and practical refernce diet C201	8
5	Mineral composition of water, diets, livers and whole bodies of Arctic charr fed semipurified reference diet C101 and practical reference diet C201	9
6	Fatty acid composition of livers and whole bodies of Arctic charr fed semipurifed reference diet C101 and practical reference diet C201	10

ABSTRACT

Yurkowski, M. 1986. Suitability of two rainbow trout (Salmo gairdneri) reference diets for Arctic charr (Salvelinus alpinus). Can. Tech. Rep. Fish. Aquat. Sci (#): iv + 10 p.

Two rainbow trout (Salmo gairdneri) reference diets (a practical reference (control) diet (C201) formulated for feed development studies and semipurified reference (basal) diet (C101) for nutrient requirement studies) were used in a 16-wk feeding trial to investigate their suitability for Arctic charr (Salvelinus alpinus). Biochemical and performance parameters together with gross morphological and histological evidence showed that diet C201 was a suitable reference diet for feed development studies in Arctic charr. It produced no pathological conditions. However diet C101 was not suitable for nutrient requirement studies in Arctic charr; compared to diet C201 it produced high mortalities, lower weight gains (growth) and low hematocrit values. It resulted in liver which was beige-gray in color, had an abnormal glycogen distribution and accumulation and had a higher weight to body weight ratio. This abnormal liver had a higher content of lipid (dry weight), moisture, calcium, iron and cobalt and a lower content of fat-free solids, copper, magnesium, and zinc. Diet C101 also produced higher whole body contents of manganese, and cobalt and lower content of selenium.

Key words: practical control diet; semipurified control diet; minerals; carbohydrates; diet development; mortality; liver glycogen accumulation; hematocrit value; basal diet.

RÉSUMÉ

Yurkowski, M. 1986. Suitability of two rainbow trout (Salmo gairdneri) reference diets for Arctic charr (Salvelinus alpinus). Can. Tech. Rep. Fish. Aquat. Sci. (#): iv + 10 p.

Deux rations alimentaires de référence de la truite arc-en-ciel (Salmo gairdneri) -- une ration de référence pratique (de contrôle) C201 établie pour des études d'élaboration de l'alimentation et une ration de référence en partie purifiée (de base) C101 établie pour des études des besoins alimentaires -- ont été servies au cours d'un essai d'alimentation de 16 semaines visant à déterminer si elles convenaient à l'omble chevalier (Salvelinus alpinus). Les paramètres biochimiques et les résultats obtenus ainsi que les indications morphologiques et histologiques brutes montrent que la ration C201 constitue un régime de référence pour les études d'élaboration de l'alimention de l'omble chevalier. Aucun cas pathologique n'y est attribuable. La ration C101, par contre, ne convenait pas aux études sur les besoins alimentaires de l'omble chevalier et, comparativement à la ration (C201), a donné lieu à beaucoup de mortalité, à des gains de poids moins importants (croissance) et à des hématocrites plus élevés. Des effets négatifs ont été notés sur le foie -- couleur beige-gris, distribution et accumulation anormales de glycogène et rapport poids du foie/poids du corps plus élevé. Le foie avait une teneur plus élevée en lipides (poids sec), en eau, en calcium, en fer et en cobalt et une teneur plus basse en solides sans graisses, en cuivre, en magnésium et en zinc. De plus, la ration C101 a également donné lieu, dans toute le corps, à des teneurs plus élevées en manganèse et en cobalt ainsi qu'à une teneur plus basse en sélénium.

Mots-clés: ration pratique de contrôle; ration de contrôle purifiée en partie; minéraux; hydrates de carbone; élaboration de l'alimentation; mortalité; accumulation de glycogène dans le foie; hématocrite; ration de base.

INTRODUCTION

Arctic charr (Salvelinus alpinus L.) is a major fish species of the Canadian Arctic and an important food source for the indigenous people. The species appears suitable for production by intensive culture (Baker 1983; Papst and Hopky 1983), but there is little information about its nutrient requirements, even in the context of empirical practical diets.

A feeding trial was undertaken to determine the suitability of two diets (C101 and C201) for nutritional studies of Arctic charr. The University of Guelph and the Ontario Ministry of Natural (OMNR) resources formulated these diets for their nutritional studies on rainbow trout (Salmo gairdneri Richardson). The practical reference diet (C201) was used in diet development studies and the semipurified (semisynthetic) reference diet (C101) for nutrient requirement studies (C.Y. Cho, OMNR, Fish Nutrition Laboratory, University of Guelph, personal communication).

Performance parameters (food consumption, weight gain, feed/gain ratios) and mortalities were measured and visual observations were made to detect pathological conditions. Because high mortalities occurred on the semipurified diet (C101) after 12 weeks of feeding, the liver and tissues were examined histologically and morphologically (gross) to determine the cause of mortality. Biochemical parameters in water, diets, liver and whole bodies, hematocrit values and liver to body mass ratios were also measured. The results would suggest necessary changes to make diet C101 suitable for nutritional studies of Arctic charr.

PROCEDURES

Arctic charr, raised at the Rockwood Experimental Hatchery at Gunton, Manitoba, from eggs collected in Labrador, were fed Silver Cup feed until the beginning of this experiment. Three replicates of 100 fish (about 310 g/100 fish ranging from 2.0-4.5 g/fish) for each of two diets were placed in tanks (30x30 cm) containing 60 L of recirculating water. System A recirculated water in one tank of fish fed diet C201 and two tanks of fish fed diet C101; system B recirculated water in two C201 and one C101 tanks (water recirculation system design see Tabachek 1983).

The two reference diets, practical reference diet C201 (formulation in Table 1), and semipurified (semisynthetic) reference diet C101 (formulation in Table 2), were fed to satiation twice daily. The diets were stored at -20°C, except for weighed portions used in each 2-wk feeding period. Feed consumption and fish weight were measured every 14 d during the 16-wk experimental period. At 12 wk, fish numbers were reduced from 100 to 50 fish/tank. Fish culled from diet C101 were fed diet C201 for 6 wk. At the end of the 16-wk period, the experiment was ended due to high mortality of fish on diet C101.

The cumulative mortalities, weight gains, feed consumption, and feed/gain ratios (dry feed consumed/wet weight gained) were calculated at the end of each 2-wk period. Liver weight as a percent of body weight, and blood hematocrit values (centrifugal sedimentation micromethod) were determined at the end of the experiment.

Lipid, fat-free solids and moisture content of diets, livers and whole bodies of Arctic charr were determined by the modified method of Folch et al. (1953). Tissues were homogenized in chloroform-methanol (2:1) mixture; the homogenate was filtered through glass microfiber filters (Whatman 934-AH) in Hirsch funnels under suction. Distilled water (0.25 vol; chloroform-washed) was added to the filtrate and mixed. The chloroform layer containing the lipids and the aqueous methanolic layer containing fat-free soluble material were recovered, and along with the fat-free insoluble residue collected on tared glass microfiber filters, were reduced to dryness. The weight of fat-free solids in the tissue is the sum of the weights of fat-free soluble material and insoluble residue in the tissue. Moisture weight in the tissue is the difference between the tissue weight and the combined weights of lipids and fat-free solids in the tissue. Moisture content of the diets was confirmed by drying at 105°C for 2 h.

The content of methionine and cysteine in the purified diet (C101) was determined by hydrolysis in 6 N HCl for 20 h; amino acids were measured by ion-exchange chromatography (Moore and Stein 1963).

The content of various minerals in water (from recirculation tanks), and in diets, liver and bodies of Arctic charr was measured. In water, the major ions (Ca, Mg, Na, K) were determined using flame atomic absorption spectroscopy (Varian Tectron Model AA-5). The minor ions (Fe, Mn, Cu, Ni, Zn, Co, Cr) were determined using flameless atomic absorption spectroscopy in combination with a carbon rod atomizer (Model CRA-90) on a Varian Tectron AA-5. Selenium was determined by the generation of selenium hydride with sodium borohydride followed by atomic absorption measurements in a heated quartz cell (Vijan and Wood 1976). Phosphorus was determined by the method of Stainton et al. (1977).

For the analysis of major and minor ions, the diet (1-2 g) was digested in nitric acid (10 mL) and perchloric acid (3 mL) until white fumes appeared. The whole fish and liver samples (2-5 g) were digested (heated) in sulfuric acid (1 mL) and nitric acid (5 mL) until the acid was driven off; the charred residue was cleared with 50% hydrogen peroxide. Major ions in the fish diets and tissue samples were assayed by flame atomic absorption spectroscopy using conditions outlined in the Varian Manual (Analytical methods for flame spectroscopy). The minor ions in the diets were assayed by the same methods used for water. Analysis of selenium in tissue was accomplished by digestion of 0.5 g samples in nitric acid and perchloric acid, followed by the same analytical procedures used for water. Phosphorus in the tissues was digested as described for trace elements and assayed by plasma

emission spectroscopy; ionization buffer used was 10% CsCl.

Niacin, riboflavin and thiamine in diets and in whole bodies of Arctic charr were determined as described by the AOAC (1970).

The composition of fatty acids in livers and in whole bodies of Arctic charr was determined by gas chromatography (Yurkowski et al. 1978); 10% SP-2300 on a 80/100 Supelcoport column was used at an oven temperature of 195°C.

Determinations of means and standard deviations of various parameters were performed with a pre-programmed calculation (Texas Instruments, Dallas, TX) and statistical significance was determined by the Student's t-test.

Gross morphological (visual) examinations were made on individual charr and tissues in search of pathological conditions. Histological examinations were made on the livers. Tissue sections were prepared by fixing the livers in Bouin's solution, embedding in paraffin, and staining for glycogen using periodic acid Schiff reagent (PAS) (Putt 1972).

RESULTS AND DISCUSSION

PRACTICAL REFERENCE DIET C201

Practical reference diet C201 fed to Arctic charr produced satisfactory growth, survival, feed consumption, and feed/gain ratios during the 16-wk feeding period (Table 3). In addition, gross morphological (visual) examination of fish, organs and tissues and histological examination of the liver revealed no apparent pathological conditions. The blood hematocrit value was 40.5±4.5% (X±SD; n=44). Liver weight was 1.38±0.24% (X±SD; n=43) of body weight, which is similar to rainbow trout with no apparent pathological conditions (NRC 1978). Content of moisture, lipid, fat-free solids, niacin, riboflavin and thiamine (liver vitamins were not assayed) in the diet C201, livers and whole bodies of Arctic charr is presented in Table 4. Content of minerals in water (from recirculation tanks), diet C201, livers and whole bodies of Arctic charr is presented in Table 5; the composition of fatty acids in the livers and whole bodies in Table 6. The data in Tables 4-6 are assumed to be within the normal range for Arctic charr because no pathological conditions were produced by diet C201. These results from the 16-wk feeding trial suggest the practical reference diet C201 is suitable for studies to develop practical diets (feeds) for Arctic charr. However, this reference diet should be tested over a longer term (at least to the first progeny stage) to confirm its acceptability. This reference diet was used to raise Labrador (20 wk; 12 to 72 g), Norway (12 wk; 13 to 93 g) and Nauyuk (24 wk;3 to 41 g) strains of Arctic charr (Tabachek 1984; Tabachek, personal communications).

SEMIPURIFIED REFERENCE DIET C101

Diet C101, compared to diet C201, supported similar growth (weight gains) in Arctic charr only during the first 12 wk, but significantly slower growth thereafter (Table 3). Fish fed diet C101 also consumed less food and had lower feed/gain ratios. Mortality rate was low for the first 12 wk, but increased dramatically, thereafter. This showed that the purified diet C101 was not a suitable reference diet for studies to determine nutrient requirements of Arctic charr. Feeding was terminated after 16 wk to determine the cause(s) of this high mortality.

Gross morphological examination showed diet C101 produced lighter colored (beige-grey) liver, and (in live fish) lighter colored skin compared to normal liver (reddish brown) and skin (dark grey) of fish on diet C201. In rainbow trout fed diets high in digestible carbohydrates, beige-grey livers are also produced (Austreng et al. 1977). Charr fed diet C101 also had low hematocrit values (20.1±9.2%; X±SD; n=42), compared to those fed diet C201 (40.5±4.5%; $P<0.01$). This may have contributed to the abnormal skin and liver color and high mortality rate (Table 3).

The abnormally-colored livers from fish fed diet C101 were found upon histological examination to have dark, dense and round granular deposits of glycogen, compared to very fine diffuse dispersions of glycogen in normal livers of fish fed diet C201. However, in fish fed diet C101 for 12 wk and then diet C201 for 6 wk, liver glycogen deposits were of intermediate density, but some dark glycogen deposits remained. Similar liver hyperglycogenesis and excessive glycogen storage was produced in rainbow trout by excess dietary digestible carbohydrates (NRC 1978). Gummy material resembling glycogen was present only in the aqueous methanol extract from abnormal livers (diet C101) and was not present in extracts from livers of charr reared on diet C201. Diets C101 and C201 contained 17% and 12% respectively of digestible carbohydrate as estimated from the content and digestibility in salmonids of carbohydrates in the ingredients (see Tables 1 and 2; NRC 1969; NRC 1978). In rainbow trout fed diet C101 to maturity, no unusual pathological conditions or excessive mortality were observed (C.Y. Cho, personnal communication). It appears that rainbow trout can tolerate about 20% digestible carbohydrate in the diet (NRC 1978), while Arctic charr can only tolerate a level somewhere between 12 and 17%.

The histologically abnormal livers (2.31±0.55% (X±SD; n=41) of body weight) found in fish fed diet C101 were also larger than normal livers (1.38±0.24% ($P<0.01$) of body weight) in fish fed diet C201. The abnormal livers also contained more lipid (only on dry weight basis) and more moisture but less fat-free solids than livers from fish fed diet C201 (Table 4). Unusually large livers with high glycogen content together with high mortality rates following high-carbohydrate dietary regimes have also been found in brook trout (Salvelinus fontinalis) (Phillips 1948), Chinook salmon (Oncorhynchus tshawytscha) (Buhler and Halver 1961), and rain-

bow trout (Austreng et al. 1977; Hickling and March 1982; Hilton and Dixon 1982; Luquet 1971; Phillips et al. 1966; NRC 1978). High levels of digestible dietary carbohydrates also caused dysfunction of rainbow trout liver (Hilton and Dixon 1982; NRC 1978) and kidney, and renal nephrosis (NRC 1978) by replacement of vital cellular structures with glycogen (NRC 1978) and perhaps also with lipids which is indicated when lipid results in Table 4 are converted to a dry weight basis. Low hematocrit values and high mortality in Arctic charr fed diet C101 may be due to impaired liver and kidney function.

The whole bodies of Arctic charr fed diets C101 and C201 contained similar concentrations of moisture, fat-free solids and lipid (Table 4). In addition, content of niacin, thiamine and riboflavin in diets and whole bodies in both lots of charr were similar (Table 4). These parameters seem not to be influenced despite the evident pathological conditions (diet C101).

It was thought that the high mortality produced by diet C101 in Arctic charr was caused by a bad lot of vitamin-free casein (major protein ingredient; see Table 2) in which the methionine and cysteine were destroyed by adverse manufacturing conditions. This methionine and cysteine deficiency was the cause of high mortality in rainbow trout (C.Y. Cho, personal communication) and in lobster (Homarus americanus) (C.H. Castell, Fisheries and Oceans Canada, Halifax, personal communication). However diet C101 contained 1.7% methionine plus cysteine which met the dietary requirements of salmonids (NRC 1978).

Differences in the content of some minerals in diets C101 and C201 as well as in livers and whole bodies of Arctic charr fed these diets (Table 5) suggests that minerals may have been involved in the production of pathological conditions by the semipurified reference diet C101. For example, diet C101 contained more calcium (7x), cobalt (56x) and copper (1.5x), but less sodium (0.5x), nickel (0.5x), iron (0.34x) and phosphorus (0.67x) than diet C201. Whole-body analysis showed that fish fed diet C101 contained more manganese and cobalt, and less selenium than fish fed diet C201. Abnormal livers in fish fed diet C101 reflected dietary concentrations in that they contained more calcium (6x) and cobalt (2x), but less magnesium (0.8x) than normal livers from fish fed diet C201. In contrast to dietary concentrations, abnormal livers contained more iron (2.5x) and less copper (0.5x) than normal livers. The results indicate an imbalance of some minerals, including calcium, copper and iron in diet C101. It appears that in Arctic charr, as in ruminants (Kirchgessner and Grassmann 1970), an excess of calcium in the diet is reflected by an accumulation of calcium in the liver. This in turn seems to promote a copper deficiency in the animal by decreasing the availability of copper from the diet, which is reflected by lower copper levels in the liver. This copper deficiency seems to cause an accumulation of iron in the liver and a corresponding decrease in the blood hematocrit values (or hemoglobin levels). In this respect the present findings in Arctic charr are similar to those described in rats (Rattus norvegicus), sheep (Ovis musimon) and pigs (Sus scrofa) (Sourkes 1970; Williams et al. 1985). In rats, copper deficiency impaired haem synthesis in the liver (Williams et al. 1985), which may explain liver iron accumulation and low hematocrit values in Arctic charr fed diet C101.

There were differences in the fatty acid composition of livers and whole bodies of Arctic charr fed diets C101 and C201 (Table 6). However, the diets did not produce essential fatty acid deficiency in these charr. This is indicated by high levels of ω6 and ω3 fatty acids and undetection of 20:3ω9 in the charr and their livers. According to Castell et al. (1972) these diets produced a ratio of 20:3ω9 to ω3 acids and ratio of 20:3ω9 to ω6 acids of less than 0.4, which indicates no deficiency in essential fatty acids. Therefore pathological conditions and high mortality in Arctic charr fed diet C101 were not caused by this deficiency.

SUMMARY AND CONCLUSIONS

1. The practical reference diet C201 was found to be a suitable reference diet for use in feed development studies of Arctic charr. However, it is recommended that this reference diet should be tested over a longer period (at least to maturity) to confirm acceptability.

2. The semipurified (semisynthetic) reference diet C101 was not found to be a suitable reference diet for use in nutrient requirement studies of Arctic charr. Fish fed this diet developed pathological accumulations of glycogen, calcium and iron in the liver, low blood hematocrit values, and a high mortality rate.

3. The results suggest that further research is needed to describe the interaction of digestible carbohydrates, calcium, copper and iron in Arctic charr. This would help explain the cause of pathological conditions produced in Arctic charr by diet C101.

ACKNOWLEDGMENTS

I thank C.Y. Cho for preparation and amino acid analysis of the purified diet C101, David Bowes for care of Arctic charr, Jo-Anne L. Tabachek for assistance during the experiment, Barnabus J. Blaskovic for technical assistance, Jim Gibson for analyses of water and Robert E. Evans for histological examination of livers.

This study was funded in part by Noval Technologies Limited, Calgary, Alberta.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC). 1970. Official methods of anal-

yses. 11th ed. W. Horwitz (ed.). AOAC, Washington, DC. 1018 p.

AUSTRENG, E., S. RISA, D.J. EDWARDS, and H. HVIDSTEN. 1977. Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. Aquaculture 11: 39-50.

BAKER, R. 1983. The effects of temperature, ration and size of the growth of Arctic charr (Salvelinus alpinus L.). M.Sc. Thesis. Univ. Manitoba, Winnipeg, MB. 227 p.

BUHLER, D.R., and J.E. HALVER. 1961. Nutrition of salmonid fishes. IX. Carbohydrate requirements of chinook salmon. J. Nutr. 74: 307-318.

CASTELL, J.D., D.J. LEE, and R.O. SINNHUBER. 1972. Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): Lipid metabolism and fatty acid composition. J. Nutr. 102: 93-100.

FOLCH, J., M. LEES, and G.H.S. STANLEY. 1953. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

HICKLING, D.R., and B.E. MARCH. 1982. A comparative study of trout and chicks regarding dietary effects on glycogen concentration in liver and muscle during feeding and subsequent to feed withdrawal. Comp. Biochem. Physiol. 72A: 115-123.

HILTON, J.W., and W.G. DIXON. 1982. Effect of increased liver glycogen and liver weight on liver function in rainbow trout, Salmo gairdneri Richardson: recovery from anesthesia and plasma ^{35}S-sulphobromophthalein clearance. J. Fish. Dis. 5: 185-195.

KIRCHGESSNER, M., and E. GRASSMANN. 1970. The dynamics of copper absorption, p. 277-287. In C.F. Mills (gen. Ed.) Trace element metabolism in animals. E. and S. Livingstone, Edinburgh.

LUQUET, P. 1971. Efficacité des proteines en relation avec leur taux d'incorporation dans l'alimentation de la truite-en-ciel. Ann. Hydrobiol. 2: 175-185.

MOORE, S., and W.H. STEIN. 1963. Chromatographic determination of amino acids by use of automatic recording equipment. p. 819-831. In S.P. Colowich and N.O. Caplan (ed.). Methods of enzymology, Vol. VI. Academic Press, New York.

NATIONAL RESEARCH COUNCIL (NRC). 1969. United States-Canadian tables of feed composition. Second Revision. National Academy of Sciences, Washington, DC. 92 p. (N.A.S.-N.R.C. Publ. 1684.)

NATIONAL RESEARCH COUNCIL (NRC). 1978. Nutrient requirements of fishes. p. 84-96. In Nutrient requirements of domestic animals. No. 10: Nutrient requirements of laboratory animals. 3rd revised ed. National Academy of Sciences, Washington, DC.

PAPST, M.H., and G.E. HOPKY. 1983. Growth of Arctic charr (Salvelinus alpinus L.) in a pilot commercial rearing system. Can. Tech. Rep. Fish. Aquat. Sci. 1182: iv + 16 p.

PHILLIPS, A.M., D.L. LIVINGSTON, and H.A. POSTON. 1966. The effect of changes in protein quality, calorie sources and calorie levels upon the growth and chemical composition of brook trout. Fish. Res. Bull. N.Y. 29: 6-14.

PHILLIPS, A.M., A.V. TUNISON, and D.R. BROCKWAY. 1948. The utilization of carbohydrate by trout. Fish. Res. Bull. N.Y. 11: 44 p.

PUTT, P.A. 1972. Manual of histopathological staining methods. John Wiley and Sons, New York. p. 145-148.

SOURKES, T.L. 1970. Factors affecting the concentration of copper in the liver of the rat, p. 247-256. In C.F. Mills (gen. ed.) Trace element metabolism in animals. E. and S. Livingstone, Edinburgh.

STAINTON, M.P., M.J. CAPEL, and F.A.J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd edition. Can. Fish Mar. Serv. Misc. Spec. Pub. 25: 166 p.

TABACHEK, J.L. 1983. Evaluation of grower diets for intensive culture of rainbow trout (Salmo gairdneri Richardson). Can. Tech. Rep. Fish. Aquat. Sci. 1178: iv + 15 p.

TABACHEK, J.L. 1984. Evaluation of grower diets for intensive culture of two strains of Arctic charr (Salvelinus alpinus L.). Can. Tech. Rep. Fish. Aquat. Sci. 1281: iv + 21 p.

VIJAN, P.N., and G.R. WOOD. 1976. Automated submicrogram determination of selenium in vegetation by quartz furnace atomic absorption spectroscopy. Talenta 23: 89-94.

WILLIAMS, D.M., KENNEDY, F.S., and GREEN, B.G. 1985. The effect of iron substrate on mitochondrial haem synthesis in copper deficiency. Brit. J. Nutr. 53: 131-136.

YURKOWSKI, M., J.K. BAILEY, R.E. EVANS, J.L. TABACHEK, G.B. AYLES, and J.G. EALES. 1978. Acceptability of rapeseed proteins in diets of rainbow trout (Salmo gairdneri). J. Fish. Res. Board Can. 35: 951-962.

Table 1. Practical reference diet (C201) formulation.[1]

Ingredients	%
Fish meal (minimum 68% protein)[2]	35.0
Soybean meal (minimum 48% protein)	20.0
Wheat middlings (minimum 17% protein)	32.4
Fish oil[3]	10.0
dl-Methionine	0.2
Vitamin premix (VIT-8004)[4]	1.0
Mineral premix (MIN-8004)[5]	1.0
Choline chloride (50% in wheat middlings)	0.4
Total	100.0

[1] University of Guelph and Ontario Ministry of Natural Resources formulation. This diet plus 40% added water was mixed and pelleted with a meat grinder modified for this purpose.

[2] Whole herring fish meal, flame-dried.

[3] Capelin oil fortified with antioxidants.

[4] Vitamin premix (10 g/Kg of feed) contains 5000 iu vitamin A (acetate); 2000 iu vitamin D_3; 200 iu vitamin E (dl-α-tocopheryl acetate); 30 mg vitamin K (menadione sodium bisulfate); 30 mg thiamine•HCl; 50 mg riboflavin; 150 mg D-calcium pantothenate; 4.0 mg biotin; 10 mg folic acid; 0.03 mg vitamin B_{12}; 200 mg niacin; 30 mg pyridoxine•HCL; 300 mg ascorbic acid; and remainder wheat middlings.

[5] Mineral premix (10 g/Kg of feed) contains 3000 g NaCl (99% NaCl); 10 mg KI (75% I); 250 mg $MnSO_4 \cdot H_2O$ (33% Mn); 300 mg $FeSO_4 \cdot 7H_2O$ (21% Fe); 100 mg $CuSO_4 \cdot 5H_2O$ (25% Cu); 400 mg $ZnSO_4 \cdot H_2O$ (36% Zn); and remainder wheat middlings.

Table 2. Semipurified reference diet (C101) formulation.[1]

Ingredients	%
Casein, vitamin-free	40
Gelatin	4
Dextrin, white	9
D-glucose (cerelose)	5
Starch	11
α-Cellulose	3
Amino acid supplement (0.5% methionine, 1.3% arginine and 0.2% starch)	2
Vitamin premix (VIT-101C)[2]	3
Mineral premix (MIN-101C)[3]	8
Fish oil[4]	15
Total	100

[1] University of Guelph and Ontario Ministry of Natural Resources formulation. This diet was prepared by steam-pelleting at 5-10 psi without water.

[2] Vitamin premix (30 g/Kg of feed) contains 7000 iu vitamin A (acetate); 3000 iu vitamin D_3; 200 iu vitamin E (dl-α-tocopheryl acetate); 50 mg vitamin K (menadione sodium bisulfate); 40 mg thiamine•HCl; 60 mg riboflavin; 200 mg D-calcium pantothenate; 0.5 mg biotin; 20 mg folic acid; 0.2 mg vitamin B_{12}; 300 mg niacin; 40 mg pyridoxine•HCl; 500 mg inositol; 400 mg ascorbic acid; 5500 mg choline chloride (separate premix for choline chloride is recommended); antioxidants (ethoxyquin, BHT or/and BHT); and α-cellulose or starch.

[3] Mineral premix (80 g/Kg of feed) contains 30 g $CaHPO_4 \cdot 2H_2O$ (23% Ca; 18%P); 3.0 g $CaCO_3$ (40% Ca); 15.0 g NaCl (39% Na); 20.0 g K_2SO4 (45% K); 10.0 g $MgSO_4$ (20% Mg). 0.7 g $FeSO_4 \cdot 7H_2O$ (21% Fe); 0.3 g $MnSO_4 \cdot H_2O$ (33% Mn); 0.55 g $ZnSO_4 \cdot H_2O$ (36% Zn); 0.16 g $CuSO_4 \cdot 5H_2O$ (25% Cu); 0.026 g $CoCl_2 \cdot 6H_2O$ (23% Co); 0.015 g KI (75% I); 0.0025 g Na_2SeO_4 (42% Se); and remainder α-cellulose or starch.

[4] Capelin oil was fortified with antioxidants.

Table 3. Performance of Arctic charr fed semipurified reference diet C101 and practical reference diet C201.

Time, weeks	Weight/100 fish, g			Weight gain/100 fish, g			Feed consumed/100 fish, g			Feed/gain ratio		Mortality/300 fish	
	C101	C201	S[1]	C101	C201	S[1]	C101	C201	S[1]	C101	C201	C101	C201
0	312±2[2]	310±2	NS										
2	450± 13	414± 9	NS	138± 12	137± 50	NS	179± 12	177± 12	NS	1.30±0.18	1.29±0.14	2	0
4	661± 25	620± 18	NS	350± 27	310± 18	NS	423± 33	483± 31	NS	1.21±0.11	1.56±0.09	4	0
6	868± 43	839± 25	NS	561± 36	529± 26	NS	713± 30	826± 41	NS	1.28±0.12	1.56±0.05	4	0
8	1133± 61	1141± 32	NS	829± 60	831± 33	NS	972± 51	1122± 33	NS	1.17±0.07	1.34±0.05	5	0
10	1469± 75	1540± 74	NS	1159± 64	1229± 74	NS	1241± 72	1492± 60	NS	1.07±0.03	1.22±0.05	6	0
12	1794± 60	1958± 75	NS	1484± 45	1655± 88	NS	1555± 68	1918± 72	*	1.05±0.02	1.15±0.03	6	1
14	1957±118	2429±103	*	1645±119	2118±104	*	1917± 76	2440±106	*	1.17±0.08	1.15±0.04	40	1
16	2189± 29	3023± 80	**	1878± 30	2711± 85	**	2175± 66	3043± 51	**	1.16±0.02	1.12±0.02	68	1

[1] S denotes significance, NS denotes $P>0.05$, * denotes $P<0.05$ and ** denotes $P<0.01$.

[2] Mean±SD of three replicates (100 fish/replicate or tank).

Table 4. Moisture, lipid, fat-free solid, protein, riboflavin, niacin and thiamine content of diets, livers[1] and whole bodies of Arctic charr fed semipurified reference diet C101 and practical reference diet C201.

Analysis	Diet		Whole body[3]			Liver[4]		
	C101[2]	C201	C101	C201	Significance[5]	C101	C201	Significance[5]
Moisture, %	8.1[6]	7.0[6]	70.5±2.0[7]	71.6±0.7[7]	NS	72.7±1.4[7]	67.8±1.4[7]	**
Solids, fat-free, %	78.8	80.7	20.3±1.0	19.2±0.8	NS	20.9±0.5	26.4±1.0	**
Lipid, %	13.2	12.3	9.2±0.8	9.2±0.8	NS	6.4±1.9	5.9±0.4	NS
Protein (Nx6.25), %	42.9	41.9						
Thiamine, mg/100 g	5.12	3.31	0.088±0.020	0.077±0.010	NS			
Riboflavin, mg/100 g	4.05	6.01	0.17±0.00	0.17±0.01	NS			
Niacin, mg/100 g	31.6	22.7	2.41±0.06	2.70±0.18	NS			

[1] Vitamins were not determined in the liver.

[2] Methionine plus cysteine content of diet C101 (% of diet; as is) is 1.72.

[3] Mean±SD (3 replicates; combined 2 fish from each replicate or tank).

[4] Mean±SD (3 replicates; combined 12 livers from each replicate or tank).

[5] NS denotes $P>0.5$, * denotes $P<0.05$ and ** denotes $P<0.01$.

[6] Moisture content in diets was determined by drying at 105°C for 2 h.

[7] Moisture determined by difference; see Procedures.

Table 5. Mineral composition of water, diets, livers and whole bodies of Arctic charr fed semipurified reference diet C101 and practical reference diet C201.

Element	Water (mg/L)[1]		Diet (µg/g)		Liver (µg/g)			Whole Body (µg/g)		
	System A[2]	System B[2]	C101	C201	C101	C201	Significance[3]	C101	C201	Significance[3]
Ca	61.3	60.4	8290	1180	495±136[4]	51±4	*	5893±331[5]	6467±892	NS
Mg	60.5	59.6	1600	1780	157±11	190±12	**	1077±21	980±79	NS
Na	41.6	42.6	2910	5700	-	-		2593±40	2517±135	NS
K	7.1	7.2	6050	6640	2603±100	2867±275	NS	12300±300	12167±666	NS
Fe	13	10	165	567	96±17	35±6	**	41±4	41±6	NS
Mn	<1	<1	85	103	1.3±0.1	1.4±0.1	NS	6.3±1.1	2.6±0.4	**
Cu	<1	<1	45.3	30.4	7.7 ±0.4	13.0±3.4	**	4.2±1.6	3.4±1.0	NS
Ni	10	9	0.8	2.1	0.17±0.06	0.40±0.20	NS	<0.50	<0.50	
Se	8	<1	1.2	0.9	0.84±0.05	0.80±0.19	NS	0.57±0.05	1.04±0.03	**
Zn	<0.5	0.7	136	197	23.1 ±1.6	27.6±1.0	**	63.4±6.9	54.1±4.4	NS
P	25	58	8680	11300	3650±352	3823±222	NS	15400±265	14133±1172	NS
Cr	<1	1	0.4	0.3	<0.01	<0.01		<0.50	<0.50	
Co	<1	<1	5.6	<0.2	0.33±0.06	<0.20		0.6±0.2	<0.2	

[1] Other water parameters (range) throughout the experiment were: temperature 11.0-11.9°C; pH, 7.60-8.26; oxygen, 9.5-10.2 mg/L; ammonia-nitrogen, 68-270 µg/L; nitrate-nitrogen 470-830 µg/L.
[2] System A was a source of recirculating water to one replicate of diet C201 and two replicates of diet C101; system B to one replicate on diet C101 and two replicates on diet C201.
[3] NS denotes P>0.05, * denotes P<0.05 and ** denotes P<0.01.
[4] Mean±SD of 3 replicates (combined 12 livers from each replicate or tank).
[5] Mean±SD of 3 replicates (combined 2 fish from each replicate or tank).

Table 6. Fatty acid composition (% weight of the total) of livers and whole bodies of Arctic charr fed semipurified reference diet C101 and practical reference diet C201.

Fatty Acid[1]	Liver			Whole Body		
	C101	C201	Significance[2]	C101	C201	Significance[2]
12:0		0.25±0.22[3]		0.44	0.06	
12:1	0.16±0.10	0.12±0.06	NS	0.18±0.16[4]	0.10±0.02	NS
13:0	0.13±0.10	0.11±0.05	NS	0.08	0.08±0.02	NS
13:1					0.02	
14:0	2.94±0.43	2.55±0.52	**	4.90±0.94	3.21±0.13	**
14:1	0.35±0.11	0.31±0.08	NS	0.65±0.07	0.30±0.01	**
15:0	0.24±0.6	0.24±0.06	NS	0.40±0.08	0.34±0.00	**
15:1	0.14±0.6	0.09±0.04	NS	0.26±0.05	0.12±0.03	**
16:0	11.98±0.71	11.98±0.58	NS	10.20±0.19	12.04±0.13	**
16:1	9.96±1.33	8.12±1.13	*	14.50±2.30	8.64±0.13	**
17:0	1.33±0.21	0.93±0.14	**	1.14±0.20	0.90±0.03	**
17:1	0.57±0.13	0.54±0.10	NS	1.02±0.16	0.74±0.08	**
18:0	3.16±0.24	2.29±0.16	**	2.20±0.15	2.10±0.13	**
18:1	29.90±2.10	26.36±1.89	*	22.20±0.39	22.13±0.38	**
18:2	1.28±0.11	4.69±0.38	**	3.33±0.71	8.20±0.10	**
18:3	0.44±0.09	0.57±0.18	**	0.89±0.16	0.89±0.16	NS
18:4	0.47±0.10	0.79±0.41	**	1.85±0.57	1.26±0.26	**
20:1	13.50±0.86	10.46±1.67	**	10.60±1.08	11.80±0.25	**
20:3ω6	0.18±0.17	0.59±0.24		0.29±0.05	0.53±0.05	**
20:4ω6[5]	0.82±0.21	1.84±0.13	**	0.51±0.11	0.92±0.04	**
20:5ω3	2.80±0.60	5.46±1.89	**	4.83±1.25	4.92±0.37	**
22:1	7.56±0.36	5.77±0.83	**	7.43±1.11	10.03±0.31	**
22:4ω6		0.01		0.16±0.03	0.14±0.09	NS
22:5ω6	0.13±0.02	0.05			0.02	
22:5ω3	0.02	0.10		0.01	0.02	
22:6ω3	11.63±0.84	15.83±2.30	**	7.60±0.71	10.02±0.20	**

[1] Shorthand notation for a fatty acid, e.g., 20:3ω6 indicates the fatty acid has 20-carbon atoms in a chain with three methylene-interrupted double bonds, and the first double bond is located 6 carbon atoms from the terminal end of the molecule.

[2] NS denotes $P>0.05$, * denotes $P<0.05$ and ** denotes $P<0.01$.

[3] Mean±SD of 3 replicates (combined 12 livers from each replicate).

[4] Mean±SD of 3 replicates (combined 2 fish from each replicate).

[5] Contains about one-third 20:3ω3.